IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Group Art Unit 1637

L.L. Smith
Electronically filed by ~~Tracy Bruesewitz~~ on April 13, 2010

In re Patent Application of

Andrei Laikhter, et al.

Application No. 10/666,998

Confirmation No.: 1003

Filed: September 19, 2003

Examiner: Mark Staples

"ANTHRAQUINONE QUENCHER DYES, THEIR METHODS OF PREPARATION AND USE"

DECLARATION OF MARK BEHLKE UNDER 37 CFR § 1.131

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Mark Behlke, do hereby declare and state the following:

1. I am an inventor of one or more claims of the above-identified invention, along with Dr. Andrei Laikhter, Dr. Yawfui Yong, Dr. Scott Rose, and Dr. Lingyan Huang.

2. I have read and understand the invention as disclosed in the present application, as defined by the presently pending claims.

3. I understand that a Request for Reconsideration is being filed in order to obtain consideration of an abstract entitled "A Novel Dark Quencher for Oligonucleotide Probes: Synthesis and Applications", authored by J.P. May et al. and distributed to attendees of the May 6-8 2002 Oligonucleotides ("TIDES") Technology Conference on May 6, 2002, was submitted to the United States Patent and Trademark Office in an Information Disclosure Statement on October 23, 2003, which the Office mistakenly failed to consider previously.

4. Prior to May 6, 2002, Dr. Laikhter synthesized mono- and di- α-aminoanthraquinone quencher phoshoramidite monomers, precursors for attaching the

quenchers to oligonucleotides, in Coralville, IA, USA. Please see Exhibit A, pages 21-25, 30-32, 34, 38, 42-44, 89, 93, and 94 from Laboratory Notebook 325 of Dr. Laikhter (dates redacted).

5. Prior to May 6, 2002, the oligonucleotides labeled with a fluorophore and with the α-aminoanthraquinone quenchers synthesized by Dr. Laikhter were used to detect a target nucleic acid sequence in a sample, according to the claimed methods. See Exhibit B, pages 67-69, 72, and 73 from Laboratory Notebook 335 of Dr. Scott Rose, dates redacted.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: April 12, 2010

Docket No. 013670-9004-US00
Q:\CLIENT\013670\9004\B2239584.1

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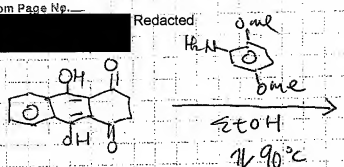

Reagents	MW	eq	Qt
Leuco-quinizarin (90%)	242.23	1	1g
Dimethoxyaniline	153.18	1.07	0.59g
EtOH	—	—	10mL

A new spot was formed. TLC showed a lot of starting material.

EXHIBIT A

To Page No.

Witnessed & Understood [redacted] Redacted

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Recorded by

Date

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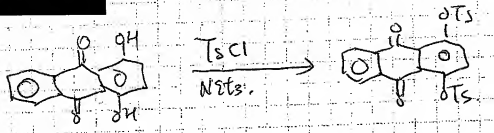

Ref JOC. Vol. 52, Pg 1307, (1987).

EXHIBIT A

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Date Redacted Invented by [redacted]

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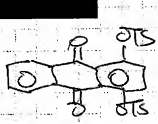

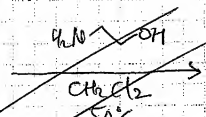

6.08
d=1.012
4AN OH

Cancelled

Reagents	eq	mw	Qt
Tosylate	1	548.59	1g
BnMgBr/ether/ CH_2Cl_2	20 eq	6.08 (d=1.012)	2.2mL / 5mL

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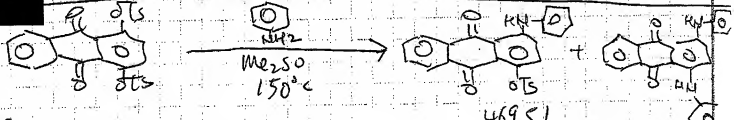
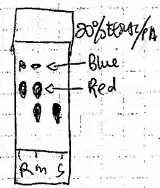


Reagents	eq	mw	Qt
Tosylate	1	548.59	1g
Bnaline	250	93.13 (d=1.022)	4ml.5g / 40mL
Me2SO			

The mixture was refluxed at 150°C for 4hrs the reaction was cooled and poured into 10% HCl (500mL). Filtration and the wash-up gave a red solid (0.65g)



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Date Redacted Invented by [redacted]
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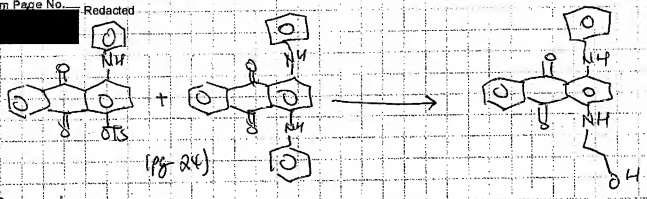

(pg 24)

Reagents	eq	mw	dt
Tosylate	1	469.51	0.658
Anhydrethanol	250	61.08 (d=1.012)	21mL
DMSO			20mL

The mixture was heated at 120°C for 3 hrs. The reaction was allowed to cool to RT and poured into 10% HCl (300mL). The thio layer was extracted with CH2Cl2 (3x), and the CH2Cl2 layer was washed with thio (1x).

Flash chromatography (5% EtOAc/PE → 100% EtOAc) gave a blue solid (0.22g)

EXHIBIT A

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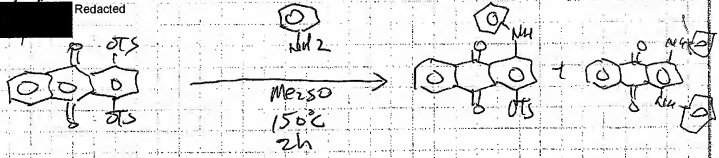

Reagents	eqs	mw	ml
Tosylate	1	548.59	39
Aniline	100	93.13 (d=1.022)	49.5 mL
Me_2SO		—	120 mL

The mixture was refluxed at 150°C for 2 hrs. The reaction was cooled and poured into 15% HCl (1500 mL). Filtration and H2O wash-up (3x) gave a reddish solid. (2.2g)

EXHIBIT A

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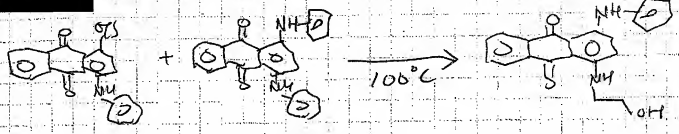

Reagents	eq	mw	wt		
Tolyltate	1	419.51	2.29		
Bromoethanol	100	61.08	~~25.2 mL~~ 30 mL	59 mL	71.5 mL
DMSO	350				

Refer to pg 25.

product = 0.6g

EXHIBIT A

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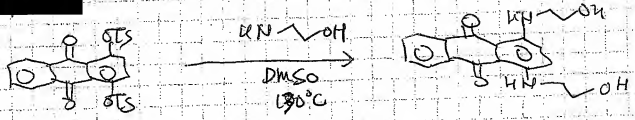

Reagent	eq	mw	qt
Tosylate	1	548.59	2.5g
Dimethylamine	250	61.08 (d=0.912)	~~79~~ 9 mL
DMSO	—	—	100 mL

the mixture was heated at 130°C for 3 hrs. the reaction mixture was cooled to RT and poured into 10% HCl (1000mL). Extraction with CH_2Cl_2 (3X) and back-extraction of the CH_2Cl_2 (organic) with H_2O (1x) @, followed by evaporation gave a blue-reddish oil. Flash Chrom (EtOAc (1L), EtOAc/CH_3CN = 1:1 Hexane, EtOAc/CH_3CN 2:8) gave the blue product. (0.18g)

EXHIBIT A

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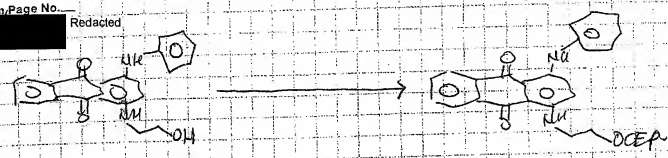

Reagents	eqv	mw	QTY
Anthragermone	1	358.39	0.3g
Chloride	1.5	236.7 (d=1.061)	~~0.23mL~~ 0.3mL
TEA	~~1.5~~ 2	101.19 (d=0.726)	0.23mL
THF	—	—	10mL

~~The mixture was stirred at 0°C~~
To a soln of anthragermone in THF, Chloride was added and TEA dropwise at 0°C. The mixture was stirred at RT for 3 hrs. The solvent was removed and the residue was dissolved in ~~EtOAc~~ EtOAc (4mL). Flash chromatography (EtOAc/PE/TEA 5/95/10 → 50/40/10) gave a blue oil. (0.35g).

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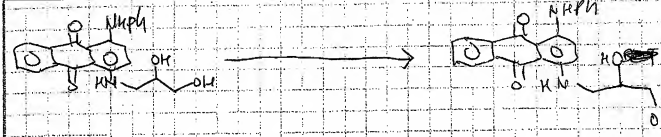

Reagent	eq	mw	Qte
Anthraquinone	1	388.42	0.3g
DMTCl	1	338.83	0.26g
Pyridine	—	—	2 mL

The mixtures was stirred at RT. After 6 hrs, TLC showed the higher spot and starting material. 1.5 eq more DMTCl was added. TLC showed complete consumption of starting material. Pyridine was removed under reduced pressure. Flash chromatography (20% EtOAc/PE → 50% EtOAc/PE) gave a blue oil (0.17g)

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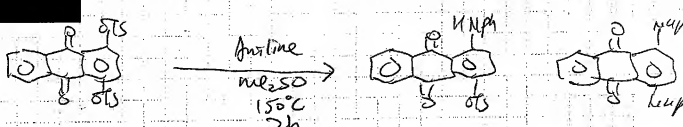

Reagents	eqs	mw	ore
Tosylate	1	469.51	50.61g
Aniline	100	61.08	68.4mL
DMSO	—	—	~~700mL~~

Refer procedure to pg 22.

product = 43g.

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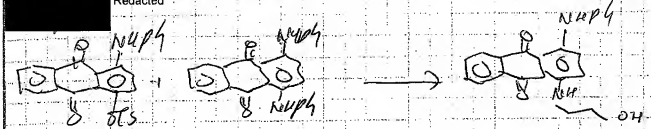

Reagents	eq	mw	QE
Anthraquinone	1	469.51	48g
Pyridoxylamine	1.00	610.8 (d=1.012)	617mL
DMSO	—	—	600mL

the reaction was heated at 110°C for 3 hrs. After cooling to RT, the mixture was poured into 10% HCl. The solid was filtered and washed with H2O. Overnite air-drying and vacuum drying gave a solid. (40g). Flash chromatography (20g solid, Biotage system), 20% EtOAc/PE — 75% EtOAc/PE. gave the desired product (5g).

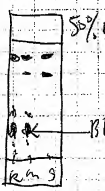

EXHIBIT A

To Page No.

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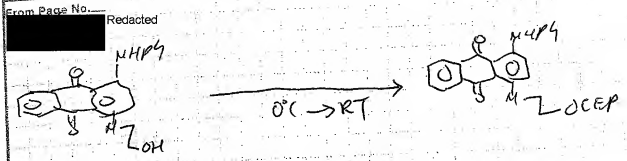

Reagent	eq	mw	use
~~Amino~~ amine	1	358.39	1g
chloride	1.5	236.7 (d=1.061)	0.93 mL
TEA	2	101.19 (d=0.726)	0.8 mL
THF	—	—	20mL

Ref exp on pg 34.

product = 1.289g

EXHIBIT A

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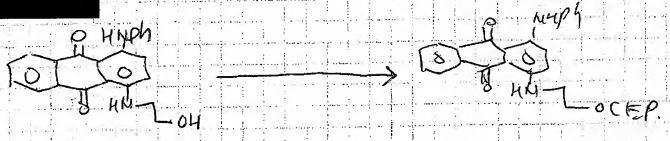

Reagents	mol.wt	eq	qty
IB	358.39	1	1g
CEP	301.43 (d=0.914)	1.2	1.1 mL
DiHT	17126	0.5	0.24g
CH_2Cl_2	—	—	20mL

The above mixtures was stirred overnite at RT. Filtration and evaporation of solvent gave a crude oil. The oil was dissolved into EtOAc (3mL) and loaded onto a flash column. Column chrom. (EtOAc/1E/TEA = 20/70/10 — 50/40/10) gave a blue oil. (1.2g).

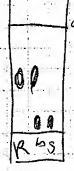

EXHIBIT A

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Witnessed & Understood by me Redacted [redacted] Date [redacted] Invented by [redacted] Date

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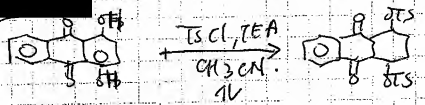

Reagents	QE
Guaiacol	50g
TsCl	96g
TEA	150mL
CH_3CN	1000mL

To guaiacol and TsCl in CH_3CN, TEA was added. The reaction was refluxed at 100°C for 6hrs. The MeCN was removed and CH_2Cl_2 (1000mL) added. The organic layer was washed with water (3x 1L) and the CH_2Cl_2 was dried over Na_2SO_4. the CH_2Cl_2 was removed and the residue was dissolved into $CHCl_3$ (1000mL) and warmed to dissolve the solid. PE (1000mL) was added slowly to ppt the brown powder. ~~After filtration~~, Filtration gave yellowish brown solid (100g).

EXHIBIT A

To Page No.

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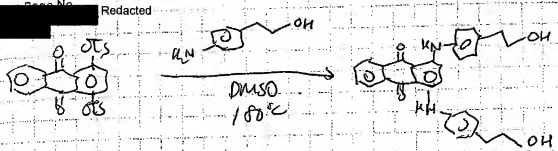
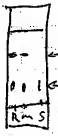
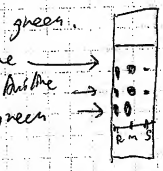


Reagent	eq	mw	qc
Tosylate	1	548.59	0.5g
Butyne	10	137.18	~~0.625g~~ 1.250g
Dmso	-	-	2mL

the mixtures was refluxed for 16 hrs. After 4hrs TLC showed the presence of a red spot.



After 16 hrs. TLC showed the disappearance of the red spot and the presence of two new spots, one blue and another green.



S= mixtures after 4hrs

1M HCl was added and stirred for 30 mins. Extraction and H2O wash gave a blue solid. Drying over vacuum gave a dry solid.

EXHIBIT A

To Page No.

Witnessed & Understood by me [redacted] Date [redacted] Recorded by [redacted] Date

From Page No. [redacted]

Real time PCR Comparison & Quenchers

MP48 Probes

		5'	3'	[μM]	
1	33490911	Fam	IB3.1	10μM	
2	30016311	IB3.1	Fam	10μM	IBHQ
3	25348900	IB.1	Fam	10μM	
4	30016341	IB2	Fam	10μM	
5	25348922	BHQ1	Fam	10μM	
6	34681371	Fam	BHQ1	34 136	20 + 252 = 10μM
7	34681381	Fam	Tamra	108	20 + 176 = 10μM
8	34681391	Tamra	Fam	93	20 + 166 = 10μM
9	36780241	Fam	dsy35	46	20 + 72 = 10μM

Primers MP48 F968 28299826 10μM
MP48 R1187 28299827 10μM

Rox SM 24846681 784 μM 5'Rox 3'Fam
~~5'~~ 5μl + 387μl H2o = 10μM
25μl @ 10μM + 475μl H2o = 500nM

Stratagene	Brilliant Plus	QPCR	ICI	
	1x	45x	1007.5	Mastermix minimum probe
H2o	14.0 M 13.5	652.5	630	(.5μl probes)
10x Cre buffer	2.5	112.5		(.1μl target)
dNTP	2.0	90		
50mM MgCl	1.5	67.5		need 10 tubes w/ 94μl MM
500μM Rox	2.5	112.5		to each tube and
Fwd primer 10μM	0.5	22.5		2μl probe (except NTC)
Rev primer 10μM	0.5	22.5		4μl target 10^6/μl
UNG	0.25	11.3		
Stratagene Taq	0.25	11.3		
	23.5			

EXHIBIT B

Witnessed & Understood by me, [redacted] Date [redacted] Invented by [redacted] Date [redacted]

5' IR3:1, Tamra, Bd1 3' Fam

EXHIBIT B

TITLE

From Pa

5' Fam 3' 183.1, Tamra, 85935

QQC

EXHIBIT B

To Page No.

Witnessed & Understood by me,

Date [redacted]

Invented by

Recorded by

Date [redacted]

From Page No. [redacted] Redacted

Repeat of station p 67 with subset of primers

		5'	3'	Stock µM
1	3349091	F	IB3.1	
2	3001635	IB3.1	F	
3	2534892	BH1	F	
4	3468137	F	BH1	
5	3468138	F	Tamra	
6	3468139	Tamra	F	
7	3678024	F	Qsy35	
8	3678025	F	Qsy9	8.5

See p67

Stratagene QPCR 1 Plus Brilliant

	1X	~~36X~~ 486	40X 540
H_2O	13.1		
10x Core buffer	2.5	~~90~~	100
dNTP (GuHCl)	2.0	~~72~~	80
@ 50 mM $MgCl_2$	1.5	~~54~~	60
500 nM Rox	2.5	~~90~~	100
For primer 10 µM	0.5	~~18~~	20
Rev primer 10 µM	0.5	~~18~~	20
UNG	0.25	9	10
SureStart Taq	0.25	9	10

Set up 9 tubes (1-8 + NTC) Probe #3349091

add 94 µl Master mix
2 µl probe (except for #8 24 µl)
4 µl target 1×10^4 copies/µl
mix

} enough for 4 wells

Add 25 µl to each well (3X)

loaded sample with P200 from bench top (NA this No target P200 p67 used no target P200 for NTC, next used P200 from bench top.

EXHIBIT B

Witnessed & Understood by me.

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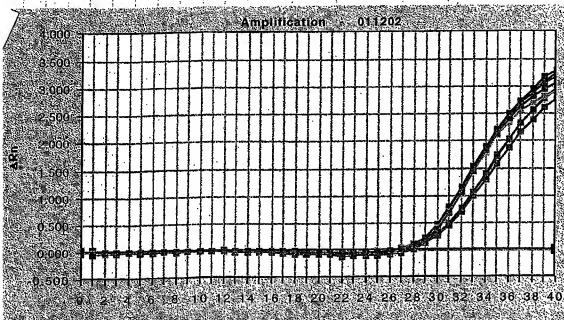
Recorded by

Date [redacted] Redacted

From Page No.

G2	UNKN	F-QSY35		26.78	0.00 0.00
G3	UNKN	F-QSY35		27.51	0.00 0.00
H1	UNKN	F-QSY7		40.00	0.00 0.00
H2	UNKN	F-QSY7		40.00	0.00 0.00
H3	UNKN	F-QSY7		40.00	0.00 0.00
A4	NTC	f-ib3.1	NTC	40.00	0.00 0.00
A5	NTC	f-ib3.1	NTC	40.00	0.00 0.00
A6	NTC	f-ib3.1	NTC	40.00	0.00 0.00

5' Fam — 3' IB3.1 - Tamra - QSY35



Samples
- FAM - A1
- FAM - A2
- FAM - A3
- FAM - E1
- FAM - E2
- FAM - E3
- FAM - G1
- FAM - G2
- FAM - G3

Viewer: ΔRn (B...
Reporter: FAM

Threshold Cycle Calculation

Threshold
Use Threshold: .038 Suggest
Multiple of Stddev: 10.0 .003
Omit Threshold: 2.0

Baseline
Start: 3 Stop: 15

Update Calculations

	Ct	Std. Dev.
FAM - A1	27.173	0.004
FAM - A2	26.798	0.003
FAM - A3	27.131	0.005
FAM - E1	27.073	0.001
FAM - E2	27.181	0.001

Same Ct
No Significant difference

EXHIBIT B

To Page No.

Witnessed & Understood by me,

Date [redacted]

Invented by

Recorded by

Date [redacted]